

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



BC

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification³ : C07C103/52; A61K 37/02 C08L 89/00	A1	(11) International Publication Number: WO 84/ 04916 (43) International Publication Date: 20 December 1984 (20.12.84)
(21) International Application Number: PCT/US84/00861 (22) International Filing Date: 5 June 1984 (05.06.84) (31) Priority Application Number: 501,321 (32) Priority Date: 6 June 1983 (06.06.83) (33) Priority Country: US (71)(72) Applicants and Inventors: COY, David, Howard [GB/US]; 4319 Perrier Street, New Orleans, LA 70115 (US). MURPHY, William, Andrew [US/US]; 601 West Hall Avenue, Slidell, LA 70458 (US). (74) Agent: TSEVDOS, Estelle, J.; American Cyanamid Company, 1937 West Main Street, P.O. Box 60, Stamford, CT 06904-0060 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>
(54) Title: CYCLIC PENTAPEPTIDES DISPLAYING SOMATOSTATIN ANTAGONISM AND METHOD OF TREATMENT OF MAMMALS THEREWITH (57) Abstract Novel cyclic pentapeptide somatostatin antagonists and method for increasing the release of growth hormone, insulin, and glucagon in mammals.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	KR	Republic of Korea
AU	Australia	LI	Liechtenstein
BE	Belgium	LK	Sri Lanka
BG	Bulgaria	LU	Luxembourg
BR	Brazil	MC	Monaco
CF	Central African Republic	MG	Madagascar
CG	Congo	MR	Mauritania
CH	Switzerland	MW	Malawi
CM	Cameroon	NL	Netherlands
DE	Germany, Federal Republic of	NO	Norway
DK	Denmark	RO	Romania
FI	Finland	SD	Sudan
FR	France	SE	Sweden
GA	Gabon	SN	Senegal
GB	United Kingdom	SU	Soviet Union
HU	Hungary	TD	Chad
JP	Japan	TG	Togo
KP	Democratic People's Republic of Korea	US	United States of America

-1-

CYCLIC PENTAPEPTIDES DISPLAYING SOMATOSTATIN
ANTAGONISM AND METHOD OF TREATMENT OF MAMMALS THEREWITH

The invention herein described relates to novel cyclic pentapeptide somatostatin antagonists and method for increasing the release of growth hormone, insulin, and glucagon in mammals therewith.

5 Mammalian somatostatin, which has the following tetradecapeptide sequence:

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Try-Lys-Thr-Phe-Thr-Ser-Cys-OH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 ,

10 inhibits the release of growth hormone from the pituitary and insulin and glucagon from the pancreatic islet cells. It also has inhibitory actions on numerous other endocrine and gastrointestinal activities in
15 mammals. These inhibitory effects are well documented (c.f., P. Brazeau *et al.*, Science 179: 77-79, (1973); W. A. Mortimer *et al.*, Lancet 1: 697-701, (1974), and C. A. Meyers *et al.*, in Gastrointestinal Hormones, edited by G. B. J. Glass, New York: Raven Press,
20 1980, pages 363-385, and their importance to the life cycle of mammals is clearly recognized.



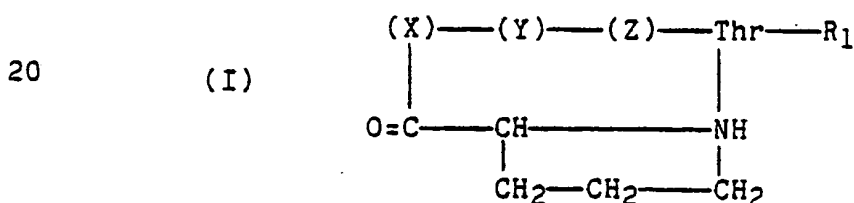
-2-

Although hundreds of somatostatin analogs have been synthesized and evaluated in an attempt to find agonists, antagonists or competitive inhibitors of said hormone, no entirely satisfactory somatostatin antagonist or competitive inhibitor of this hormone has been available or disclosed in the art.

It is, therefore, an object of the present invention to provide a novel somatostatin antagonist effective for increasing release of growth hormone, insulin, and glucagon in mammals.

Surprisingly, this objective has been achieved with the synthesis of cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)], and derivatives and pharmaceutically acceptable salts thereof.

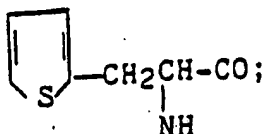
The novel somatostatin antagonists of this invention are cyclic pentapeptides depicted by formula (I) and the pharmaceutically acceptable salts thereof, having the following amino acid sequence:



wherein X is phenylalanyl, tyrosyl, tryptophyl, 4-chlorophenylalanyl, 4-bromophenylalanyl, 4-fluorophenylalanyl, histidyl, 5-fluorotryptophyl, 5-chlorotryptophyl, 5-bromotryptophyl, 5-methoxytryptophyl, 5-methyltryptophyl, 4-methoxyphenylalanyl, 4-benzyloxyphenylalanyl, 4-methylphenylalanyl, or



-3-



Y is D-tryptophyl, D-phenylalanyl, D-4-chlorophenylalanyl, D-4-bromophenylalanyl, D-4-fluorophenylalanyl, D-4-methoxyphenylalanyl, D-4-benzyloxyphenylalanyl, D-4-methylphenylalanyl, D-tyrosyl, D-5-fluorotryptophyl, D-5-bromotryptophyl, D-5-chlorotryptophyl, D-5-methoxytryptophyl, D-5-methyltryptophyl or D-histidyl; Z is lysyl or arginyl; Thr is threonyl and R₁ is hydrogen, benzyl or substituted benzyl, connected to the threonyl amino acid residue through its 3-oxygen atom.

The R₁ group referred to above may thus be illustrated as follows:



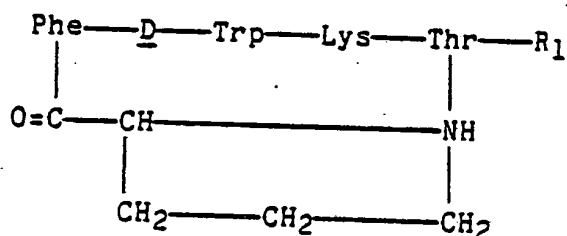
wherein R₂ is hydrogen, halogen (including fluorine, chlorine, bromine and iodine, C₁-C₄ alkoxy (preferably methoxy) or C₁-C₄ alkyl (preferably methyl)).

Preferred compounds of the invention have the formula (I) structure wherein R₁ is hydrogen or benzyl; Z is lysyl, and X and Y represent chiral amino acid residues selected from those described for X and Y above.



-4-

An especially preferred cyclic pentapeptide somatostatin antagonist of this invention is the cyclo[Pro-Phe-D-Trp-Lys-Thr(R₁)] which may be illustrated as follows:



wherein Phe is phenylalanyl; D-trp is D-tryptophyl; Lys is lysyl; Thr is threonyl, and R₁ is hydrogen or benzyl.

The term "pharmaceutically acceptable salts," as used in the present specification, is intended to mean non-toxic acid addition salts which are commonly used in the pharmaceutical industry. Among those of particular interest in the present invention are the hydrochloride, phosphate, sulfate, laurate, citrate, gluconate, succinate; acetate, and oleate salts of the formula (I) compounds. These salts are readily prepared by methods well known in the art.

In keeping with standard nomenclature, abbreviations for chiral amino acid residues used in the present specification and claims are as follows:

<u>Abbreviation</u>	<u>Name</u>
Phe	= L-phenylalanyl
Tyr	= L-tyrosyl
Trp	= L-tryptophyl
(4-Cl)Phe	= L-4-chlorophenylalanyl
(4-Br)Phe	= L-4-bromophenylalanyl
(4-F)Phe	= L-4-fluorophenylalanyl
His	= L-histidyl



-5-

	<u>Abbreviation</u>		<u>Name</u>
	(4-CH ₃)Phe	=	<u>L</u> -4-methylphenylalanyl
	(4-CH ₃ O)Phe	=	<u>L</u> -4-methoxyphenylalanyl
	(4-Bzl-O)Phe	=	<u>L</u> -4-benzyloxyphenylalanyl
	(5-F)Trp	=	<u>L</u> -5-fluorotryptophyl
5	(5-Cl)Trp	=	<u>L</u> -5-chlorotryptophyl
	(5-Br)Trp	=	<u>L</u> -5-bromotryptophyl
	(5-CH ₃ O)Trp	=	<u>L</u> -5-methoxytryptophyl
	(5-CH ₃)Trp	=	<u>L</u> -5-methyltryptophyl
	<u>D</u> -(4-F)Phe	=	<u>D</u> -4-fluorophenylalanyl
10	<u>D</u> -(4-Cl)Phe	=	<u>D</u> -4-chlorophenylalanyl
	<u>D</u> -(4-Br)Phe	=	<u>D</u> -4-bromophenylalanyl
	<u>D</u> -(4-CH ₃)Phe	=	<u>D</u> -4-methylphenylalanyl
	<u>D</u> -(4-CH ₃ O)Phe	=	<u>D</u> -4-methoxyphenylalanyl
	<u>D</u> -(4-Bzl-O)Phe	=	<u>D</u> -4-benzyloxyphenylalanyl
15	<u>D</u> -Phe	=	<u>D</u> -phenylalanyl
	<u>D</u> -Tyr	=	<u>D</u> -tyrosyl
	<u>D</u> -Trp	=	<u>D</u> -tryptophyl
	<u>D</u> -(5-F)Trp	=	<u>D</u> -5-fluorotryptophyl
	<u>D</u> -(5-Br)Trp	=	<u>D</u> -5-bromotryptophyl
20	<u>D</u> -(5-Cl)Trp	=	<u>D</u> -5-chlorotryptophyl
	<u>D</u> -(5-CH ₃ O)Trp	=	<u>D</u> -5-methoxytryptophyl
	<u>D</u> -(5-CH ₃)Trp	=	<u>D</u> -5-methyltryptophyl
	<u>D</u> -His	=	<u>D</u> -histidyl
	Lys	=	<u>L</u> -lysyl
25	Arg	=	<u>L</u> -arginyl
	Ala	=	<u>L</u> -alanyl
	Gly	=	<u>L</u> -glycyl
	Cys	=	<u>L</u> -cysteinyl
	Asn	=	<u>L</u> -asparginyl
30	Ser	=	<u>L</u> -seryl
	Pro	=	<u>L</u> -prolyl



-6-

Other abbreviations used in the present specification are:

Bzl = benzyl
FMOC = fluorenylmethyloxycarbonyl
Boc = t-butyloxycarbonyl
HPLC = high performance liquid chromatography
TFA = trifluoroacetic acid
TLC = thin-layer chromatography

In accordance with the process of the present invention, solid-phase synthesis of the formula (I) peptides can be carried out on a Beckman 990 automatic peptide synthesizer. Preparative HPLC can be performed on a thick-walled glass column (2.5 x 45 cm) containing Whatman LRP-1 reverse phase packing (C₁₈ silica 13-20 μ m) pumped with Fluid Metering Company pump and pulse damper; and amino acid analyses can be run on a Beckman 119 CL analyzer and processed with a System AA computing integrator.

Amino acid derivatives utilized in the preparation of the compounds of the present invention are available from several chemical supply houses including: Bachem, Inc., Torrance, California; and Chemical Dynamics, Inc., Plainfield, New Jersey.

Conveniently, the formula (I) peptides of this invention, cyclo[Pro-(X)-(Y)-(Z)-Thr-R₁], can be prepared beginning with the appropriate α -amino protected amino acid coupled to an appropriate resin, such as a polystyrene resin with one-two percent by weight of divinyl benzene as a cross-linking agent.



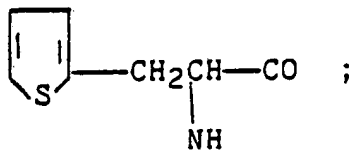
-7-

Protecting groups for the amino acids include: t-butyloxycarbonyl, isopropylloxycarbonyl, diisopropylloxycarbonyl, benzyloxycarbonyl, cyclopentylloxycarbonyl, and the like; but, t-butyloxycarbonyl or isopropylloxycarbonyl are generally preferred.

5 In the process, FMOC-(Z)-[Boc-Thr(Bzl)]-Pro-(X)-(Y)-O-CH₂-resin is prepared beginning with the appropriate Boc-(Y)-Merrifield resin, where Y is D-Trp, D-Phe, D-Tyr, D-(5-F)Trp, D-(5-Br)Trp, D-(5-Cl)Trp, D-(5-OCH₃)Trp, D-(5-CH₃)Trp, D-(4-F)Phe, D-(4-Cl)Phe, D-(4-Br)Phe, D-(4-CH₃)Phe, D-(4-CH₃O)Phe, D-(4-Bzl-O)Phe, or D-His. The resin is placed in the reaction vessel of the peptide synthesizer which has been programmed to carry out the following work-wash cycle: (a) methylene chloride, (b) 33% TFA in methylene chloride, (c) 10 methylene chloride, (d) ethyl alcohol, (e) methylene chloride, (f) 10% triethylamine in methylene chloride, and (g) methylene chloride.

15 The washed resin with the Boc group removed is then stirred with t-Boc-(X), where X is Phe, Tyr, Trp, (4-Cl)Phe, (4-Br)Phe, (4-F)Phe, (4-CH₃)Phe, (4-CH₃O)-Phe, (4-Bzl-O)Phe, (5-F)Trp, (5-Cl)Trp, (5-Br)Trp, (5-CH₃O)Trp, (5-CH₃)Trp, His or

25

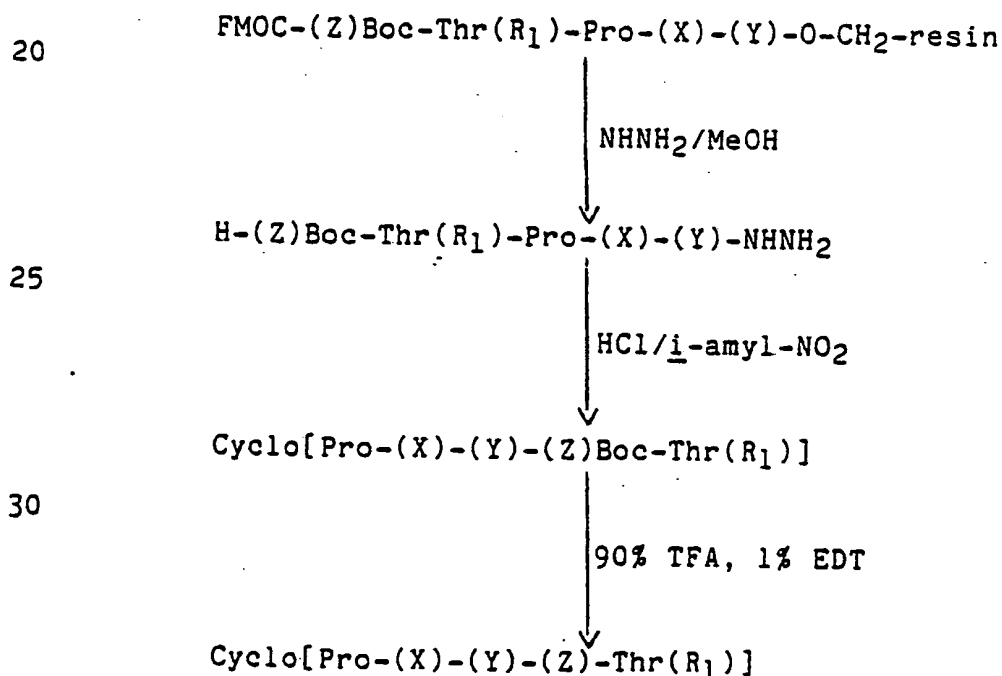


30



-8-

and diisopropylcarbodiimide in methylene chloride. The protected attached amino acids are then cycled through steps (b) through (g) in the above wash program. Thereafter, the following amino acids are coupled by the same cycle of events: t-Boc-Pro, t-Boc-Thr(R₁) and Fmoc-(Z)-Boc, wherein R₁ is benzyl or substituted benzyl, and Z is Lys or Arg. These steps provide the Fmoc-(Z)Boc-Thr(R₁)-Pro-(X)-(Y)-O-CH₂-resin. Removal of the protected peptide from the resin by treatment with a ten-fold excess of hydrazine in methanol is accompanied by loss of the base-sensitive Fmoc group to give H(Z)Boc-Thr(R₁)-Pro-(X)-(Y)NHNH₂, which is cyclized with hydrochloric acid and isoamylnitrite to give cyclo[Pro-(X)-(Y)-(Z)-Boc-Thr(R₁)]. Treatment of this peptide with 90% TFA in water containing 1% ethanedithiol then yields the desired somatostatin antagonist of this invention cyclo-[Pro-(X)-(Y)-(Z)-Thr(R₁)]. These reactions are graphically illustrated in the Flow Diagram I below:

FLOW DIAGRAM I

-9-

Alternatively, the compounds of this invention may also be prepared by the process hereinafter described.

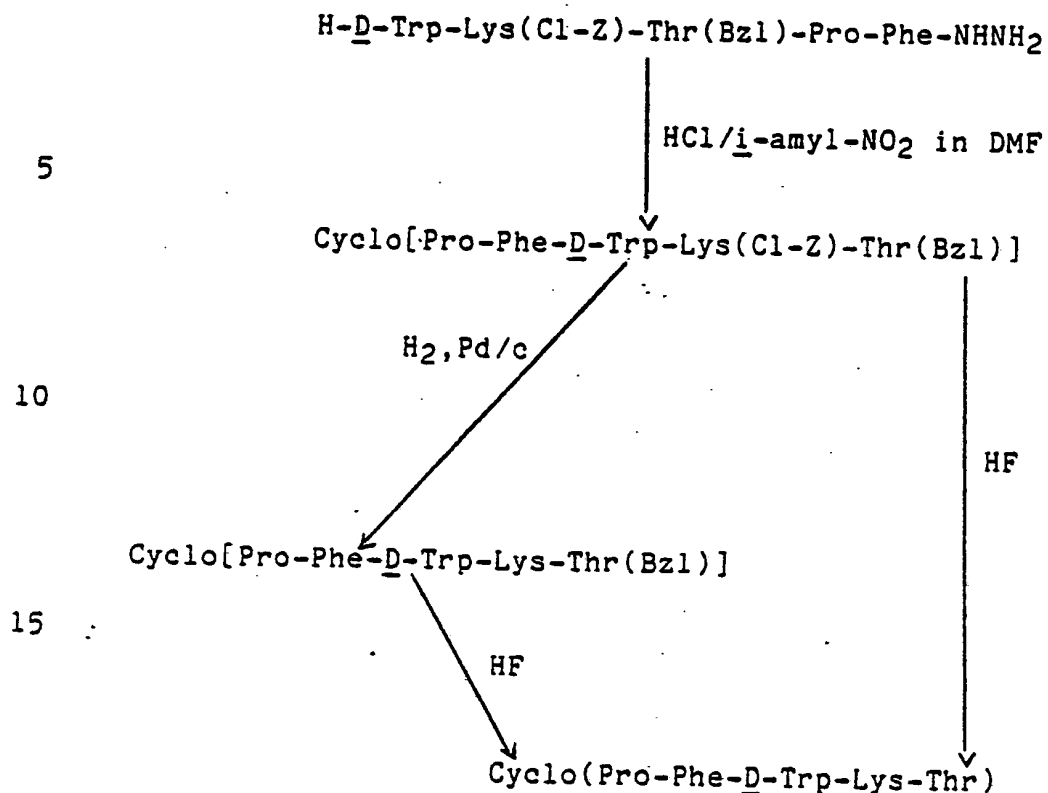
After conventional solid-phase assembly beginning with 1 mmole of Boc-Phe-Merrifield resin (1% cross-linked), the protected peptide hydrazide, H-D-Trp-Lys(Cl-Z)-Thr(Bzl)-Pro-Phe-NHNH₂, is cleaved from the support by treatment with hydrazine in methanol and isolated by a procedure described by D. F. Veber *et al.*, Proceedings of the National Academy of Science, U.S.A., 75: 2636-2640, (1978). Without further purification, the hydrazide is converted to the azide, neutralized with triethylamine, and cyclized in DMF solution to yield the protected ninhydrin negative material, cyclo[Pro-Phe-D-Trp-Lys-(Cl-Z)-Thr(Bzl)]. When this protected ninhydrin negative material is hydrogenated at atmospheric pressure and room temperature over 10% Pd on charcoal, the compound, cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)], is formed and found to be a very effective somatostatin antagonist. When, however, the protected crude ninhydrin negative material is deprotected by treatment with HF-anisole under standard conditions, as described by G. T. Engberg *et al.*, Nature, 293: 222-223, (1981), and purified by preparative HPLC eluting with 10-55% gradient of CH₃CN in 20% AcOH, the reaction yields the pure peptide, cyclo(Pro-Phe-D-Trp-Lys-Thr), which has somatostatin antagonist activity.

It is also found that HF treatment of the active somatostatin antagonist, cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)], converts it to the peptide, cyclo(Pro-Phe-D-Trp-Lys-Thr), which also has somatostatin antagonist activity. The above reactions are graphically illustrated in Flow Diagram II below.



-10-

FLOW DIAGRAM II



20 Among the cyclic pentapeptides of the present invention that display somatostatin antagonism and can be prepared by the process described and illustrated in Flow Diagram II above, are:

25 Cyclo[Pro-Phe-D-Tyr-Arg-Thr(Bzl)];
Cyclo[Pro-His-D-(5-F)Trp-Lys-Thr(Bzl)];
Cyclo[Pro-(4-F)Phe-D-Trp-Lys-Thr(3-F-Bzl)];
Cyclo[Pro-Phe-D-(5-CH₃)Trp-Lys-Thr(Bzl)];
Cyclo[Pro-Tyr-D-Tyr-Lys-Thr(Bzl)];
30 Cyclo[Pro-Phe-D-His-Lys-Thr(Bzl)];
Cyclo[Pro-Trp-D-Phe-Arg-Thr(Bzl)];
Cyclo[Pro-(4-Br)Phe-D-(5-Br)Phe-Lys-Thr(Bzl)];
Cyclo[Pro-Tyr-D-His-Lys-Thr(Bzl)];
Cyclo[Pro-Tyr-D-His-Arg-Thr(Bzl)];
35 Cyclo[Pro-Trp-D-Tyr-Lys-Thr(3-CH₃-Bzl)];



-11-

- Cyclo[Pro-Phe-D-(4-F)Phe-Lys-Thr(Bzl)];
Cyclo[Pro-Phe-D-(4-Cl)Phe-Lys-Thr(Bzl)];
Cyclo[Pro-Phe-D-(4-Br)Phe-Lys-Thr(Bzl)];
Cyclo[Pro-Phe-D-(4-CH₃)Phe-Lys-Thr(Bzl)];
Cyclo[Pro-Phe-D-(4-CH₃O)Phe-Lys-Thr(Bzl)];
5 Cyclo[Pro-Phe-D-(4-Bzl-O)Phe-Lys-Thr(Bzl)];
Cyclo[Pro-(4-Me)Phe-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-(4-CH₃O)Phe-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-(4-Bzl-O)Phe-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)];
10 Cyclo[Pro-Phe-D-Trp-Arg-Thr(Bzl)];
Cyclo[Pro-Phe-D-Trp-Lys-Thr(4-Cl-Bzl)];
Cyclo[Pro-Phe-D-Trp-Lys-Thr(4-OCH₃-Bzl)];
Cyclo[Pro-Phe-D-Trp-Lys-Thr(4-CH₃-Bzl)];
Cyclo[Pro-Phe-D-Trp-Arg-Thr(3-Br-Bzl)];
15 Cyclo[Pro-Tyr-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-Trp-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-(4-Cl)Phe-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-(4-F)Phe-D-Trp-Lys-Thr(Bzl)];
Cyclo[Pro-His-D-Tyr-Lys-Thr(Bzl)];
20 Cyclo[Pro-Trp-D-(5-OCH₃)Trp-Lys-Thr(Bzl)];
Cyclo[Pro-(4-Cl)Phe-D-Tyr-Arg-Thr(4-OCH₃-Bzl)];
Cyclo[Pro-Phe-D-Phe-Lys-Thr(Bzl)];
Cyclo[Pro-His-D-Phe-Lys-Thr(4-Cl-Bzl)];
Cyclo[Pro-Phe-D-(5-OCH₃)Trp-Lys-Thr(Bzl)], and
25 Cyclo[Pro-(4-Br)Phe-D-Tyr-Arg-Thr(Bzl)].



-12-

- Cyclo(Pro-Phe-D-Tyr-Arg-Thr);
Cyclo[Pro-His-D-(5-F)Trp-Lys-Thr];
Cyclo[Pro-Phe-D-(5-CH₃)Trp-Lys-Thr];
Cyclo(Pro-Tyr-D-Tyr-Lys-Thr);
Cyclo(Pro-Phe-D-His-Lys-Thr);
5 Cyclo(Pro-Trp-D-Phe-Arg-Thr);
Cyclo[Pro-(4-Br)Phe-D-(5-Br)Phe-Lys-Thr];
Cyclo(Pro-Tyr-D-His-Lys-Thr);
Cyclo(Pro-Tyr-D-His-Arg-Thr);
Cyclo[Pro-Phe-D-(4-F)Phe-Lys-Thr];
10 Cyclo[Pro-Phe-D-(4-Cl)Phe-Lys-Thr];
Cyclo[Pro-Phe-D-(4-Br)Phe-Lys-Thr];
Cyclo[Pro-Phe-D-(4-CH₃)Phe-Lys-Thr];
Cyclo[Pro-Phe-D-(4-CH₃O)Phe-Lys-Thr];
Cyclo[Pro-Phe-D-(4-Bzl-O)Phe-Lys-Thr];
15 Cyclo[Pro-(4-Me)Phe-D-Trp-Lys-Thr];
Cyclo[Pro-(4-CH₃O)Phe-D-Trp-Lys-Thr];
Cyclo[Pro-(4-Bzl-O)Phe-D-Trp-Lys-Thr];
Cyclo(Pro-Phe-D-Trp-Lys-Thr);
Cyclo(Pro-Phe-D-Trp-Arg-Thr);
20 Cyclo(Pro-Tyr-D-Trp-Lys-Thr);
Cyclo(Pro-Trp-D-Trp-Lys-Thr);
Cyclo[Pro-(4-Cl)Phe-D-Trp-Lys-Thr];
Cyclo[Pro-(4-F)Phe-D-Trp-Lys-Thr];
Cyclo(Pro-His-D-Tyr-Lys-Thr);
25 Cyclo[Pro-Trp-D-(5-OCH₃)Trp-Lys-Thr];
Cyclo(Pro-Phe-D-Phe-Lys-Thr);
Cyclo[Pro-Phe-D-(5-OCH₃)Trp-Lys-Thr], and
Cyclo[Pro-(4-Br)Phe-D-Tyr-Arg-Thr].



-13-

5 The compounds of formula (I) are useful as somatostatin antagonists and are effective for increasing the release of growth hormone, insulin, and glucagon, in mammalian hosts, when administered thereto at dosages of from 0.000002 to 1 mg/kg of mammalian body weight per day.

10 The formula (I) compounds may be administered orally in the form of a feed additive or as a bolus, pill, tablet, oral gel, or the like, designed to deliver the active compound at the dosage level desired. They may also be administered parenterally by intramuscular, subcutaneous, intraperitoneal or intravenous injection, or as a transdermal or nasal spray.

15 As will hereinafter be shown, injection of formula (I) peptide into rats blocks the inhibitory effects of exogenous somatostatin on growth hormone, insulin, and glucagon release. In practice, it is also found that in fasted rats, basal hepatic portal insulin and glucagon levels are significantly increased after treatment with cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)]; and, 20 further, that plasma growth hormone levels in NEMBUTAL®-anesthetized and stimulated rats are increased after injection with the above-said compound.

25 The following examples further illustrate the present invention, but are not intended to be limitative thereof.

30

35 NEMBUTAL® sodium pentobarbital is a product of Abbott Laboratories.



-14-

EXAMPLE 1Preparation of N^α9-fluorenylmethyloxycarbonyl-N^ε-tert-butylloxycarbonyl-L-lysyl-O-benzyl-L-threonyl-L-protyl-L-phenylalanyl-D-tryptophyl-O-CH₂ resin

5 t-Butyloxycarbonyl-D-tryptophan-Merrifield resin (1% cross-linked, 2.5 g, 1.0 mmole) is placed in the reaction vessel of a Beckman Model 990 automatic peptide synthesizer programmed to carry out the following work-wash cycle: (a) CH₂Cl₂; (b) 33% trifluoroacetic acid in
10 CH₂Cl₂ (two times for one and 25 minutes each); (c) CH₂Cl₂; (d) C₂H₅OH; (e) CH₂Cl₂; (f) 10% (C₂H₅)₃N in CH₂Cl₂ (two times for two minutes each); (g) CH₂Cl₂.

 The washed resin with the t-butyloxycarbonyl (Boc) group removed is stirred with t-butyloxycarbonyl-phenylalanine (t-Boc-Phe) and diisopropylcarbodiimide
15 (3 mmole) in CH₂Cl₂ for one hour and the resulting amino acid resin then washed with CH₂Cl₂. The protected, attached amino acids are then cycled through steps (b) through (g) in the above wash program. The following
20 amino acids (3 mmole) are then coupled successively by the same treatment cycle: t-butyloxycarbonyl-prolyl (t-Boc-Pro); t-butyloxycarbonyl-threonyl-benzyl [t-Boc-Thr(Bzl)] and fluorenylmethyloxycarbonyl-lysyl-t-butyloxycarbonyl [FMOC-Lys(Boc)].

25 Upon completion of the last coupling reaction, the resulting resin is washed with methanol to obtain the above-identified resinated product.



-15-

EXAMPLE 2Preparation of N ϵ -tert-butyloxycarbonyl-L-lysyl-O-benzyl-L-threonyl-L-prolyl-L-phenylalanyl-D-tryptophyl-NHNH₂

N α 9-fluorenylmethyloxycarbonyl-N ϵ -tert-butyloxycarbonyl-L-lysyl-O-benzyl-L-threonyl-L-prolyl-L-phenylalanyl-D-tryptophyl-O-CH₂ resin (1.87 g, 0.5 mmol) is suspended in 70 ml of dry methanol and the thus prepared suspension then treated with 7 ml of anhydrous hydrazine. The mixture is stirred for 24 hours at ambient temperature and then filtered, washed with methanol and the methanol extracts evaporated to yield an oil that solidified upon tritoraction with ether to give 0.65 g of the above-named product in the form of a white powder.

EXAMPLE 3

15 Preparation of cyclo(L-prolyl-L-phenylalanyl-D-tryptophyl-N ϵ -tert-butyloxycarbonyl-L-lysyl-O-benzyl-L-threonyl)

The peptide hydrazide N ϵ -tert-butyloxycarbonyl-L-lysyl-O-benzyl-L-threonyl-L-prolyl-L-phenylalanyl-D-tryptophyl-NHNH₂ (0.3 g, 0.33 mmol) is dissolved in 10 ml of dry dimethylformamide (DMF) and cooled to -35°C. The solution is then acidified to pH 1.5 with hydrochloric acid in tetrahydrofurfuryl alcohol (HCl/THF) and isoamyl nitrite (67 μ l). The mixture is stirred at -35°C for 45 minutes and then added to 1.5 ml of dimethylformamide (DMF) and cooled to -25°C. The reaction mixture is then neutralized to pH 7.5 with diisopropylethylamine and maintained at -25°C for 24 hours and 5°C for an additional 24 hours. Evaporation of the DMF gives the above-identified product as a brown oil that exhibits a major spot by thin-layer chromatography (tlc).



-16-

EXAMPLE 4Preparation of cyclo(L-prolyl-L-phenylalanyl-D-tryptophyl-L-lysyl-O-benzyl-L-threonyl)

5 The fully protected peptide cyclo(L-prolyl-L-phenylalanyl-D-tryptophyl-N^ε-tert-butyloxycarbonyl-L-lysyl-O-benzyl-L-threonyl) is dissolved in 50 ml of a mixture of trifluoroacetic acid, water, and 1,2-ethanedithiol prepared in a ratio of 45:5:2 by volume. The solution is stirred at ambient temperature for 40 minutes and then evaporated to an oil. The resulting oil is
10 dissolved in 50% acetic acid and eluted on a column (2.5 x 95 cm) of Sephadex G-15 with 50% acetic acid. Lyophilized material from a major peak absorbing at 280 nm is then applied to a column of Whatman LRP-1 octadecylsilanesilica and eluted with a linear gradient
15 of 10 to 15% acetonitrile in 20% acetic acid solution.

The major peak is collected and lyophilized to give the required peptide as a white, fluffy powder (90 mg). This material gives one Cl-starch, ninhydrin and Ehrlich reagent-positive spot with the following
20 R_f's on silica gel TLC plates: n-BuOH:AcOH:H₂O:EtOAc (1:1:1:1), 0.68; n-BuOH:AcOH:H₂O (4:1:1), 0.47; EtOAc:pyridine:AcOH:H₂O (5:5:1:3), 0.79; 2-PrOH:1MAcOH (2:1), 0.61. Amino acid analysis of a sample hydrolyzed in
25 methanesulfonic acid containing 0.1% tryptamine gives: Thr, 0.98; Pro, 1.01; Lys, 0.97; Trp, 0.85.



-17-

EXAMPLE 5Preparation of cyclo(L-prolyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl)

5 The dried peptide, cyclo(L-prolyl-L-phenylalanyl-D-tryptophyl-L-lysyl-O-benzyl-L-threonyl), is placed in a vessel suitable for HF cleavage. This vessel also contains a magnetic stirring bar. A small quantity of anisole sufficient to wet the peptide is added to this vessel. The vessel is next connected to an HF line and placed under vacuum to remove any air
10 therein. The vessel is then cooled to about -78°C with a dry ice-acetone bath. Doubly distilled HF (about 10 ml/gm of peptide) is added to the vessel. The dry ice-acetone bath is then removed from the vessel and replaced by an ice-water bath. The vessel's contents
15 are vigorously stirred for about 45 minutes while the vessel remains immersed in the ice-water bath. Most of the HF in the vessel is then removed by water aspiration. After the majority of HF is removed by water aspiration, the remaining HF and anisole are removed via a vacuum
20 pump to afford the title peptide.



-18-

EXAMPLE 6

Evaluation of peptide effects on growth hormone, insulin, and glucagon release in mammals using the rat as the test species

5 In this evaluation, the procedures described by W. A. Murphy *et al.*, Endocrinology, 109:491-495 (1980), were employed.

10 In growth hormone (GH) experiments, male rats (Charles Rivers) were anesthetized with NEMBUTAL® (5 mg per 100 g/BW) which also served to maintain stimulated plasma GH levels. Exactly 30 minutes after the rats were anesthetized, 0.5 ml of saline or the test peptide in saline was administered as a sc bolus. A 1 ml blood sample was drawn from the jugular vein 15 minutes after the injection. For examining blocking effects of the
15 analog on somatostatin, the cyclic peptide was given five minutes prior to somatostatin. GH levels were determined using NIADDKD rat GH RIA components.

Data obtained are reported in Table I below.



-19-

TABLE I

Effects of the Cyclic Pentapeptides, the Compounds of the Present Invention, Cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)] and the Unprotected Cyclo(Pro-Phe-D-Trp-Lys-Thr) Compared To Somatostatin on GH Levels in the Rat (Five Animals Per Group unless otherwise given).

	Peptide	Dose (μ g/100 g body weight)	GH (ng/ml)*
10	A) Saline	-	389 \pm 60 (6)
	B) Somatostatin	0.4	152 \pm 51 (5)
	C) Cyclo(Pro-Phe-D-Trp-Lys-Thr)	2	730 \pm 110 (6)
15	D) Cyclo(Pro-Phe-D-Trp-Lys-Thr)	10	459 \pm 100 (5)
	C + B		75 \pm 29 (5)
	D + B		114 \pm 36 (4)

	A) Saline	-	648 \pm 177
20	B) Cyclo(Pro-Phe-D-Trp-Lys-Thr)	0.3	1265 \pm 318
	C) Cyclo(Pro-Phe-D-Trp-Lys-Thr)	0.6	1072 \pm 187
	D) Cyclo(Pro-Phe-D-Trp-Lys-Thr)	1.2	728 \pm 143
25	-----		
	A) Saline	-	497 \pm 80
	B) Somatostatin	0.5	173 \pm 46
	C) Cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)]	0.2	1724 \pm 393
30	D) Cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)]	0.6	812 \pm 163
	C + B		149 \pm 73
	D + B		370 \pm 73

* = Mean \pm standard error (n).



-20-

From the above data, it can be seen that the compound of the present invention and an unprotected relative thereof were evaluated to determine the effects of both peptides on NEMBUTAL®-stimulated GH release in rats. Radioimmunoassayable GH levels in control-stimulated jugular blood 648 ± 177 ng/ml showed an increase to 1265 ± 318 after injection with 0.3 μ g/100 g body weight of the unprotected analog cyclo(Pro-Phe-D-Trp-Lys-Thr); whereas, the radioimmunoassayable GH levels in control-stimulated jugular blood of 497 ± 80 ng/ml rose significantly to 1724 ± 393 ng/ml after injection of 0.2 g/100 g body weight of the compound of the invention, i.e., the protected compound cyclo[Pro-Phe-D-Trp-Lys- Thr(Bzl)].

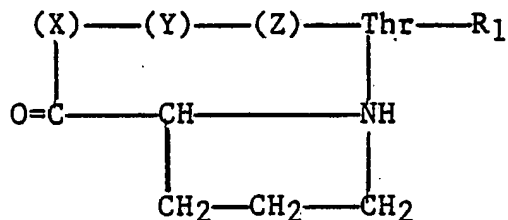
From the above data, it appears that the antagonist effect of the compounds of the present invention is not wholly dependent on the presence of the aromatic benzyl protecting group on the threonine.



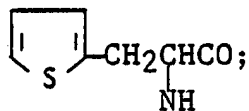
-21-

WHAT IS CLAIMED IS:

1. A cyclic pentapeptide compound having the structural formula:



wherein X is phenylalanyl, tyrosyl, tryptophyl, 4-chlorophenylalanyl, 4-bromophenylalanyl, 4-fluorophenylalanyl, 4-methoxyphenylalanyl, 4-benzyloxyphenylalanyl, 4-methylphenylalanyl, 5-fluorotryptophyl, 5-chlorotryptophyl, 5-bromotryptophyl, 5-methoxytryptophyl, 5-methyltryptophyl, histidyl or



Y is D-tryptophyl, D-5-fluorotryptophyl, D-5-bromotryptophyl, D-5-chlorotryptophyl, D-5-methoxytryptophyl, D-5-methyltryptophyl, D-phenylalanyl, D-4-chlorophenylalanyl, D-4-bromophenylalanyl, D-4-fluorophenylalanyl, D-4-methoxyphenylalanyl, D-4-benzyloxyphenylalanyl, D-4-methylphenylalanyl, D-tyrosyl or D-histidyl; Z is lysyl or arginyl; Thr is threonyl and R₁ is hydrogen, benzyl or substituted benzyl connected to the threonyl amino acid residue through its 3-oxygen atom; or a pharmaceutically acceptable salt thereof.



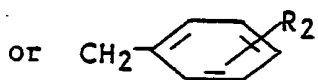
-22-

2. A compound according to Claim 1, wherein R_1 is hydrogen or benzyl; Z is lysyl and X and Y represent chiral amino acid residues selected from those described for X and Y in said Claim 1.

3. A compound according to Claim 1, cyclo[Pro-Phe-D-Trp-Lys-Thr(R_1)] wherein R_1 is hydrogen or



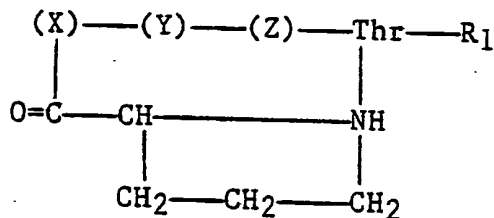
and R_2 is H, F, Cl, Br, I, C_1 - C_4 alkoxy or C_1 - C_4 alkyl; or cyclo-[Pro-Phe-D-Trp-Arg-Thr(R_1)] wherein R_1 is hydrogen



and R_2 is H, F, Cl, Br, I, C_1 - C_4 alkoxy or C_1 - C_4 alkyl.

4. A compound according to Claim 1, cyclo-[Pro-Phe-D-Trp-Lys-Thr(Bzl)]; or cyclo(Pro-Phe-D-Trp-Lys-Thr).

5. A method for increasing release of growth hormone, insulin and glucagon, in mammals comprising administering thereto from 0.00001 to 1 mg/kg of mammalian body weight/day of a compound having the structural formula:

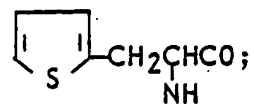


wherein X is phenylalanyl, tyrosyl, tryptophyl, 4-chlorophenylalanyl, 4-bromophenylalanyl, 4-fluoro-



-23-

phenylalanyl, 4-methoxyphenylalanyl, 4-benzyloxyphenylalanyl, 4-methylphenylalanyl, 5-fluorotryptophyl, 5-chlorotryptophyl, 5-bromotryptophyl, 5-methoxytryptophyl, 5-methyltryptophyl, histidyl or



Y is D-tryptophyl, D-5-fluorotryptophyl, D-5-bromotryptophyl, D-5-chlorotryptophyl, D-5-methoxytryptophyl, D-5-methyltryptophyl, D-phenylalanyl, D-4-chlorophenylalanyl, D-5-bromophenylalanyl, D-4-fluorophenylalanyl, D-4-methoxyphenylalanyl, D-4-benzyloxyphenylalanyl, D-4-methylphenylalanyl, D-tyrosyl, or histidyl; Z is

lysyl or arginyl; R₁ is hydrogen or $-\text{CH}_2 - \text{C}_6\text{H}_4 - \text{R}_2$;

R₂ is H, Cl, F, Br, I, C₁-C₄ alkoxy or C₁-C₄ alkyl; or pharmaceutically acceptable salts thereof.

6. A method according to Claim 5 for releasing growth hormone in mammals, wherein the compound administered to said mammalian host is cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)]; or cyclo(Pro-Phe-D-Trp-Lys-Thr).

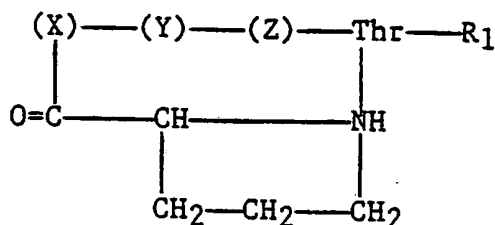
7. A method according to Claim 5 for releasing insulin in mammals, wherein the compound administered to said mammalian host is cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)]; or cyclo(Pro-Phe-D-Trp-Lys-Thr).

8. A method according to Claim 5 for releasing glucagon in mammals, wherein the compound administered to said mammalian host is cyclo[Pro-Phe-D-Trp-Lys-Thr(Bzl)]; or cyclo(Pro-Phe-D-Trp-Lys-Thr).

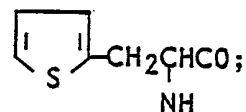


-24-

9. A method for the preparation of a cyclic pentapeptide compound having the structural formula:



wherein X is phenylalanyl, tyrosyl, tryptophyl, 4-chlorophenylalanyl, 4-bromophenylalanyl, 4-fluorophenylalanyl, 4-methoxyphenylalanyl, 4-benzyloxyphenylalanyl, 4-methylphenylalanyl, 5-fluorotryptophyl, 5-chlorotryptophyl, 5-bromotryptophyl, 5-methoxytryptophyl, 5-methyltryptophyl, histidyl or



Y is D-tryptophyl, D-5-fluorotryptophyl, D-5-bromotryptophyl, D-5-chlorotryptophyl, D-5-methoxytryptophyl, D-5-methyltryptophyl, D-phenylalanyl, D-4-chlorophenylalanyl, D-4-bromophenylalanyl, D-4-fluorophenylalanyl, D-4-methoxyphenylalanyl, D-4-benzyloxyphenylalanyl, D-4-methylphenylalanyl, D-tyrosyl or D-histidyl; Z is lysyl or arginyl; Thr is threonyl and R_1 is hydrogen, benzyl or substituted benzyl connected to the threonyl amino acid residue through its 3-oxygen atom; or a pharmaceutically acceptable salts thereof; said method characterized by reacting an amino acid protected by an α -amino acid protecting group, coupled to a resin with about 1% to 2% by weight, of a cross-linking agent.



-25-

10. A method according to Claim 9, wherein the amino acid protecters are t-butyloxycarbonyl, isopropylcarbonyl, diisopropyloxycarbonyl, benzyloxycarbonyl, cyclopentylonycarbonyl; the resin is polystyrene resin; and the cross-linking agent is divinyl benzene.



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US84/00861

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. C07C 103/52; A61K 37/02; C08L 89/00 U. S. Cl. 260-112.5R; 260-112.5S; 424-177; 525-54.11																							
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁴</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; text-align: left;">Classification System</th> <th style="border: 1px solid black; text-align: left;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">U. S.</td> <td style="border: 1px solid black; padding: 5px;">260-112.5R; 260-112.5S; 424-177;</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵</div>			Classification System	Classification Symbols	U. S.	260-112.5R; 260-112.5S; 424-177;																	
Classification System	Classification Symbols																						
U. S.	260-112.5R; 260-112.5S; 424-177;																						
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black; text-align: left;">Category [*]</th> <th style="width: 60%; border: 1px solid black; text-align: left;">Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷</th> <th style="width: 30%; border: 1px solid black; text-align: left;">Relevant to Claim No. ¹⁸</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">US, A, 4,235,886 (Freidinger) 25 November 1980</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">9-10</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">US, A, 4,101,721 (Rich), 18 July 1978</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">9-10</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">A</td> <td style="border: 1px solid black; vertical-align: top;">N, J. Am. Chem. Soc., Vol. 85, 1963, Merrifield, Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide, pages 2149-2154</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">9-10</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">A</td> <td style="border: 1px solid black; vertical-align: top;">N, Chemical Abstracts, Vol. 80, 1974, Yoshioka, Molecular Structure of Cyclochlorotine, A Toxic Chlorine-Containing Cyclic Pentapeptide, page 460, Abstract No. 83616f</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-3</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">A</td> <td style="border: 1px solid black; vertical-align: top;">N, Int. J. Peptide Protein Res., Vol. 21, 1983, Nutt, Somatostatin Analogs which Define The Role of The Lysine-9 Amino Group, pages 66-73</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">A</td> <td style="border: 1px solid black; vertical-align: top;">N, Chemical Abstracts, Vol. 97, 1982, Yoshitomi, Cyclic Pentapeptide Derivatives, page 876, Abstract No. 182876y</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> </table> <div style="font-size: small; margin-top: 10px;"> <p>[*] Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div>			Category [*]	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸	X	US, A, 4,235,886 (Freidinger) 25 November 1980	9-10	X	US, A, 4,101,721 (Rich), 18 July 1978	9-10	A	N, J. Am. Chem. Soc., Vol. 85, 1963, Merrifield, Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide, pages 2149-2154	9-10	A	N, Chemical Abstracts, Vol. 80, 1974, Yoshioka, Molecular Structure of Cyclochlorotine, A Toxic Chlorine-Containing Cyclic Pentapeptide, page 460, Abstract No. 83616f	1-3	A	N, Int. J. Peptide Protein Res., Vol. 21, 1983, Nutt, Somatostatin Analogs which Define The Role of The Lysine-9 Amino Group, pages 66-73	1-8	A	N, Chemical Abstracts, Vol. 97, 1982, Yoshitomi, Cyclic Pentapeptide Derivatives, page 876, Abstract No. 182876y	1-8
Category [*]	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸																					
X	US, A, 4,235,886 (Freidinger) 25 November 1980	9-10																					
X	US, A, 4,101,721 (Rich), 18 July 1978	9-10																					
A	N, J. Am. Chem. Soc., Vol. 85, 1963, Merrifield, Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide, pages 2149-2154	9-10																					
A	N, Chemical Abstracts, Vol. 80, 1974, Yoshioka, Molecular Structure of Cyclochlorotine, A Toxic Chlorine-Containing Cyclic Pentapeptide, page 460, Abstract No. 83616f	1-3																					
A	N, Int. J. Peptide Protein Res., Vol. 21, 1983, Nutt, Somatostatin Analogs which Define The Role of The Lysine-9 Amino Group, pages 66-73	1-8																					
A	N, Chemical Abstracts, Vol. 97, 1982, Yoshitomi, Cyclic Pentapeptide Derivatives, page 876, Abstract No. 182876y	1-8																					
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search ² <div style="border-top: 1px solid black; margin-top: 5px;">23 August 1984</div> International Searching Authority ¹ <div style="border-top: 1px solid black; margin-top: 5px;">ISA/US</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report ³ <div style="border-top: 1px solid black; margin-top: 5px;">27 AUG 1984</div> Signature of Authorized Officer ¹⁰ <div style="border-top: 1px solid black; margin-top: 5px;"> </div> </td> </tr> </table>			Date of the Actual Completion of the International Search ² <div style="border-top: 1px solid black; margin-top: 5px;">23 August 1984</div> International Searching Authority ¹ <div style="border-top: 1px solid black; margin-top: 5px;">ISA/US</div>	Date of Mailing of this International Search Report ³ <div style="border-top: 1px solid black; margin-top: 5px;">27 AUG 1984</div> Signature of Authorized Officer ¹⁰ <div style="border-top: 1px solid black; margin-top: 5px;"> </div>																			
Date of the Actual Completion of the International Search ² <div style="border-top: 1px solid black; margin-top: 5px;">23 August 1984</div> International Searching Authority ¹ <div style="border-top: 1px solid black; margin-top: 5px;">ISA/US</div>	Date of Mailing of this International Search Report ³ <div style="border-top: 1px solid black; margin-top: 5px;">27 AUG 1984</div> Signature of Authorized Officer ¹⁰ <div style="border-top: 1px solid black; margin-top: 5px;"> </div>																						

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	N, J. Am. Chem. Soc., Vol. 104, No. 2, 1982, Bach, Determination of Proline Ring Nonplanarity from Proton Spin-Spin Coupling Constants: Applications to Two Cyclic Pentapeptides, pages 572-576	1-3
---	---	-----

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:
2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

THIS PAGE BLANK (USPTO)